PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Tapas Mukhopadhyay, *et al.*	Group Art Unit: 1642
Serial No.: 10/043,877	Examiner: B. J. Fetterolf
Filed: January 9, 2002	Atty. Dkt. No.: INRP:095US
For: ANTIHELMINTHIC DRUGS AS A TREATMENT FOR HYPERPROLIFERATIVE DISEASES	

SECOND DECLARATION OF TAPAS MUKHOPADHYAY, SUNIL CHADA, ABNER MHASHILKAR, AND JACK A. ROTH UNDER 37 C.F.R. §1.131

We, Tapas Mukhopadhyay, Sunil Chada, Abner Mhashilkar, and Jack A. Roth, hereby declare as follows:

1. We are the joint inventors of the subject matter claimed in the above-referenced patent application, U.S.S.N. 10/043,887, filed January 9, 2002.

2. We are submitting this declaration to set forth facts demonstrating that we both conceived the idea of the invention as reflected in the claims of the above-referenced patent application and determined that it functioned, prior to March 9, 1999.

3. Submitted as Exhibit 1 to this declaration is a copy of a FACS assay showing our experiments and results, entitled figures "1A" and "1B" which was prepared prior to March 9, 1999.

Tapas Mukhopadhyay

4. Submitted as Exhibit 2 to this declaration is a copy of our experiments and results in a study of the treatment of p53 wild type lung cancer cells with fenbendazole, which took place prior to March 9, 1999.

5. Exhibit 1 shows the results of our cell cycle analysis involving A549 (p53 wild type) non-small cell lung cancer (NSCLC) cells that have been treated with fenbendazole. The results show that the untreated A549 cells (A549C), have a standard profile of cells in various phases of the cell cycle, G1/S/G2, indicating a dominant G1 population. In contrast, the fenbendazole treated cells (A549 7EN) show a depression of both G2 and S phases and a G1 block. Furthermore, the fenbebdazole treated cells show a distinct sub-G0-G1 population indicative of apoptotic cells. We generated the results of this cell cycle analysis prior to March 9, 1999.

6. Exhibit 2 shows the results of our study of the treatment of p53 wild type lung cancer with fenbendazole. We determined that treatment of p53 wild type lung cancer cells with fenbendazole inhibits growth. The study evaluated growth of lung cancer cells or normal lung epithelium (NHBEC) after treatment with fenbendazole (labeled FEN in the figure) and other agents. Both H1299 and H322 are p53 deficient NSCLC cells and show modest growth inhibition by fenbendazole after 5-7 days. In contrast, the p53 wild type cells A549 and H460 show dramatic inhibition of cell growth by fenbendazole that is evident by day 1-3 and 50-80% growth inhibition by day 5-7 of treatment. The control normal cells, NHBEX do not show growth inhibition by fenbendazole. We generated the results of this study prior to March 9, 1999.

7. All work disclosed in the invention disclosure form was conducted in the United States of America.

8. Therefore, the invention as reflected in the claims of the above-referenced patent application was reduced to practice prior to March 9, 1999.

9. We hereby declare that all statements made by our own knowledge are true and all statements made on information and belief are believed to be true and further that statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment under § 100 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date	Tapas Mukhopadhyay
Date	Sunil Chada
Date	Abner Mhashilkar
Date	Jack A. Roth

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6. Exhibit 2 shows the results of our study of the treatment of p53 wild type lung cancer with fenbendazole. We determined that treatment of p53 wild type lung cancer cells with fenbendazole inhibits growth. The study evaluated growth of lung cancer cells or normal lung epithelium (NHBEC) after treatment with fenbendazole (labeled FEN in the figure) and other agents. Both H1299 and H322 are p53 deficient NSCLC cells and show modest growth inhibition by fenbendazole after 5-7 days. In contrast, the p53 wild type cells A549 and H460 show dramatic inhibition of cell growth by fenbendazole that is evident by day 1-3 and 50-80% growth inhibition by day 5-7 of treatment. The control normal cells, NHBEX do not show growth inhibition by fenbendazole. We generated the results of this study prior to March 9, 1999.

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Date	Tapas Mukhopadhyay
07/21/05 Date	Sunil Chada
Date	Abner Mhashilkar
Date	Jack A. Roth

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6. Exhibit 2 shows the results of our study of the treatment of p53 wild type lung cancer with fenbendazole. We determined that treatment of p53 wild type lung cancer cells with fenbendazole inhibits growth. The study evaluated growth of lung cancer cells or normal lung epithelium (NHBEC) after treatment with fenbendazole (labeled FEN in the figure) and other agents. Both H1299 and H322 are p53 deficient NSCLC cells and show modest growth inhibition by fenbendazole after 5-7 days. In contrast, the p53 wild type cells A549 and H460 show dramatic inhibition of cell growth by fenbendazole that is evident by day 1-3 and 50-80% growth inhibition by day 5-7 of treatment. The control normal cells, NHBEX do not show growth inhibition by fenbendazole. We generated the results of this study prior to March 9, 1999.

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Date	Tapas Mukhopadhyay
Date	Sunil Chada
08/10/05 Date	Abhijit Mhashilkar
Date	Jack A. Roth

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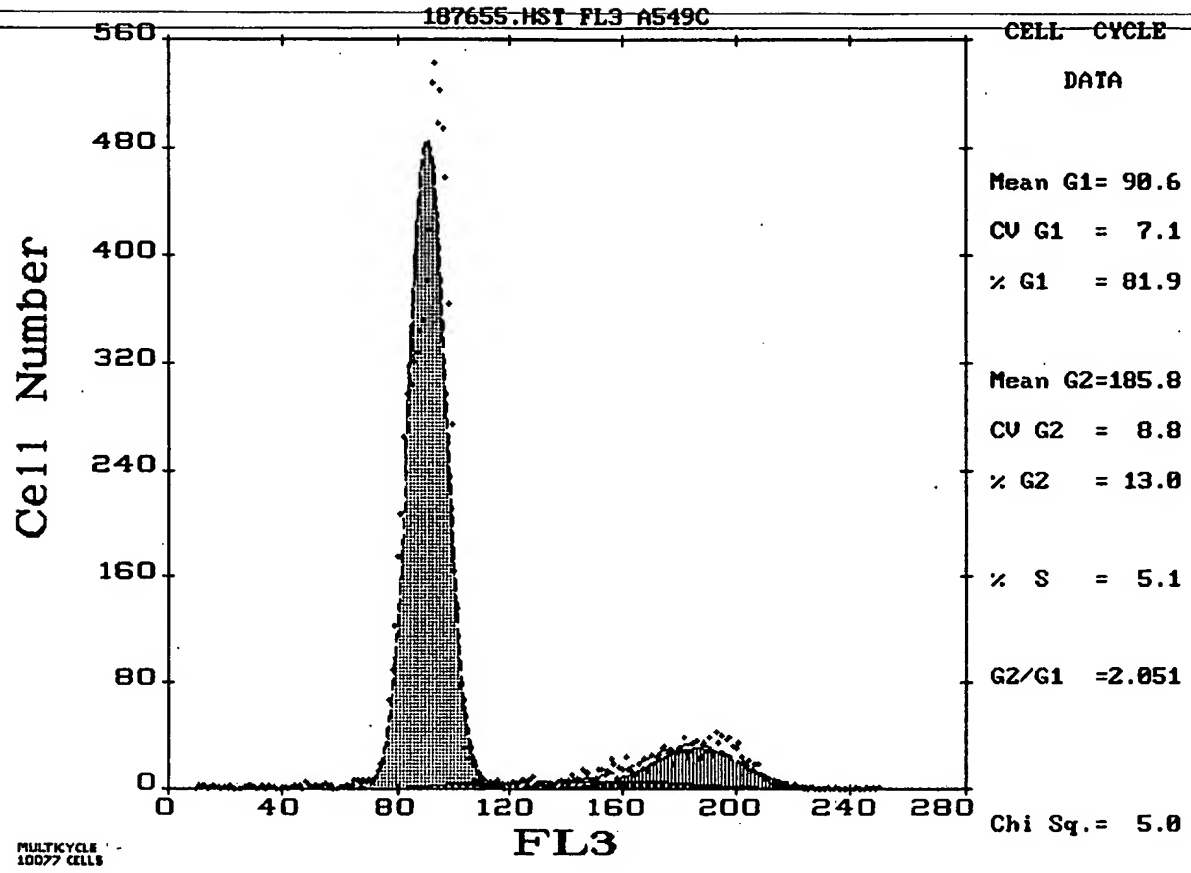
Date	Tapas Mukhopadhyay
Date	Sunil Chada
Date	Abner Mhashilkar
8-15-05 Date	Jack A. Roth

Exhibit 1

(1) A


187655.HST FL3 A549C
Cell Number
560
480
400
320
240
160
80
0
0
40
80
120
160
200
240
280
FL3
MULTICYCLE
10077 CELLS
CELL CYCLE DATA
Mean G1= 90.6
CV G1 = 7.1
% G1 = 81.9
Mean G2=185.8
CV G2 = 8.8
% G2 = 13.0
% S = 5.1
G2/G1 =2.051
Chi Sq.= 5.0

(1) B

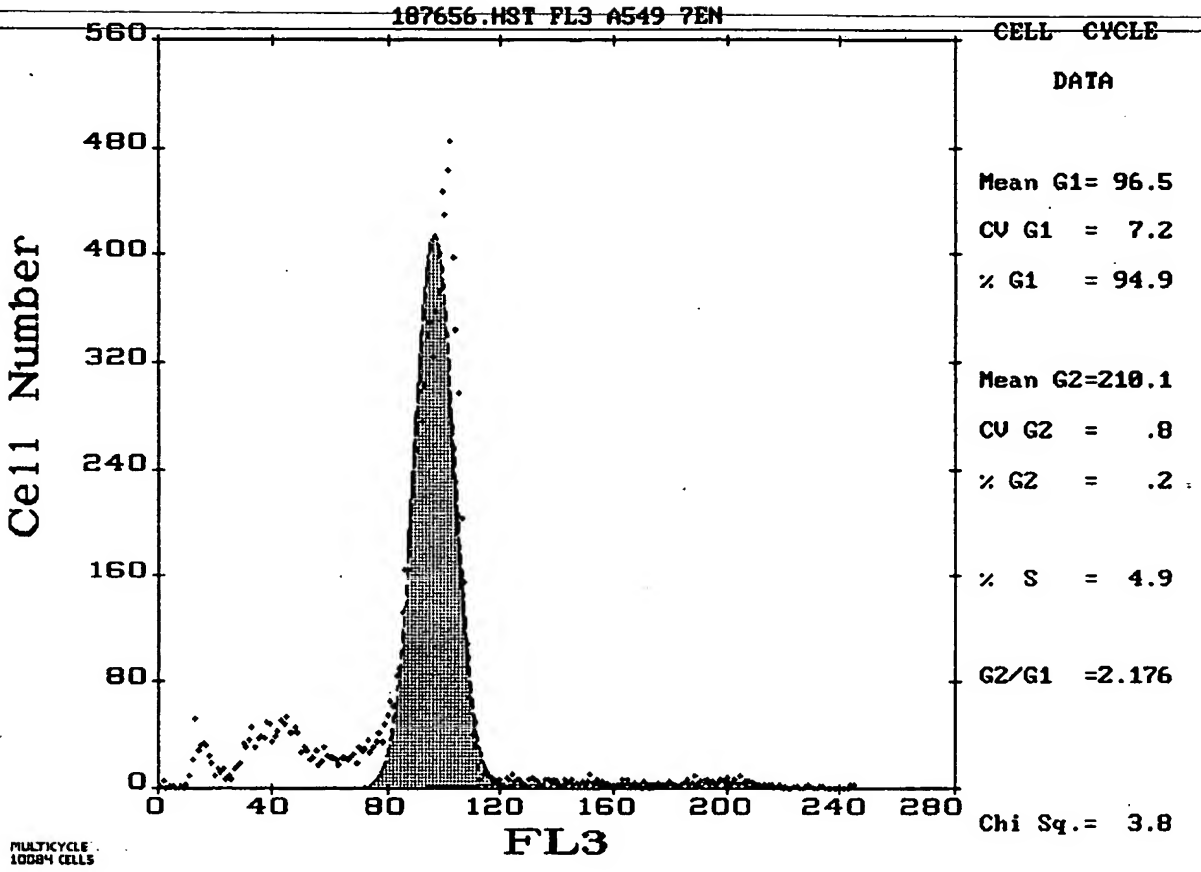
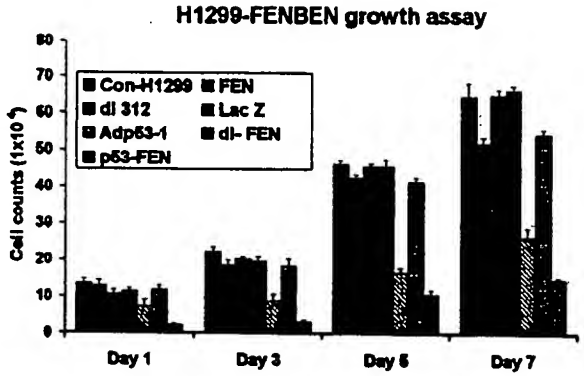
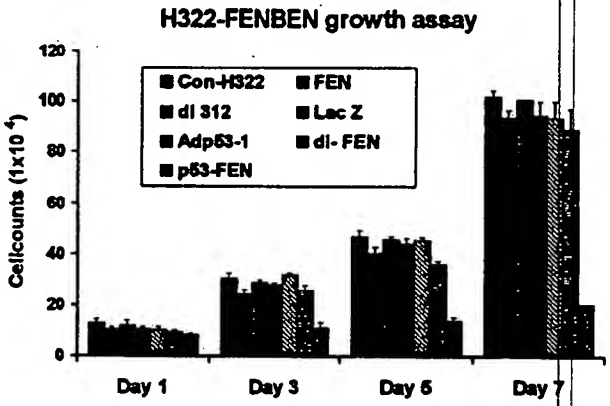
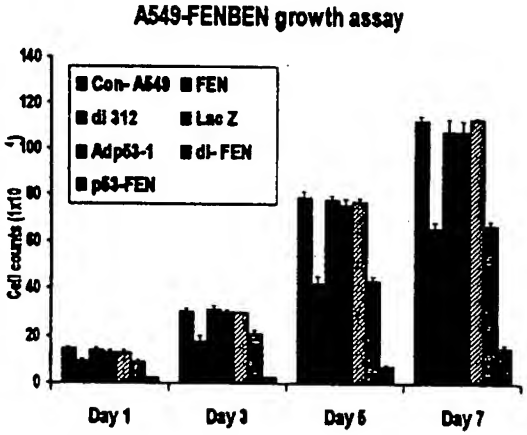
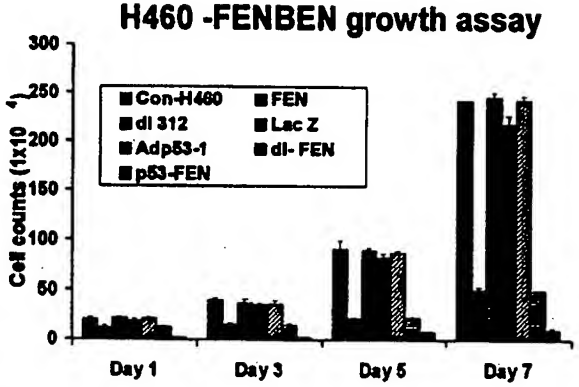

187656.HST FL3 A549 7EN
Cell Number
560
480
400
320
240
160
80
0
0
40
80
120
160
200
240
280
FL3
MULTICYCLE
10084 CELLS
CELL CYCLE
DATA
Mean G1= 96.5
CV G1 = 7.2
% G1 = 94.9
Mean G2=210.1
CV G2 = .8
% G2 = .2
% S = 4.9
G2/G1 =2.176
Chi Sq.= 3.8

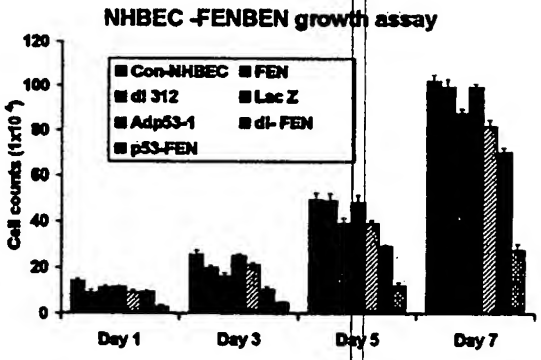
Exhibit 2

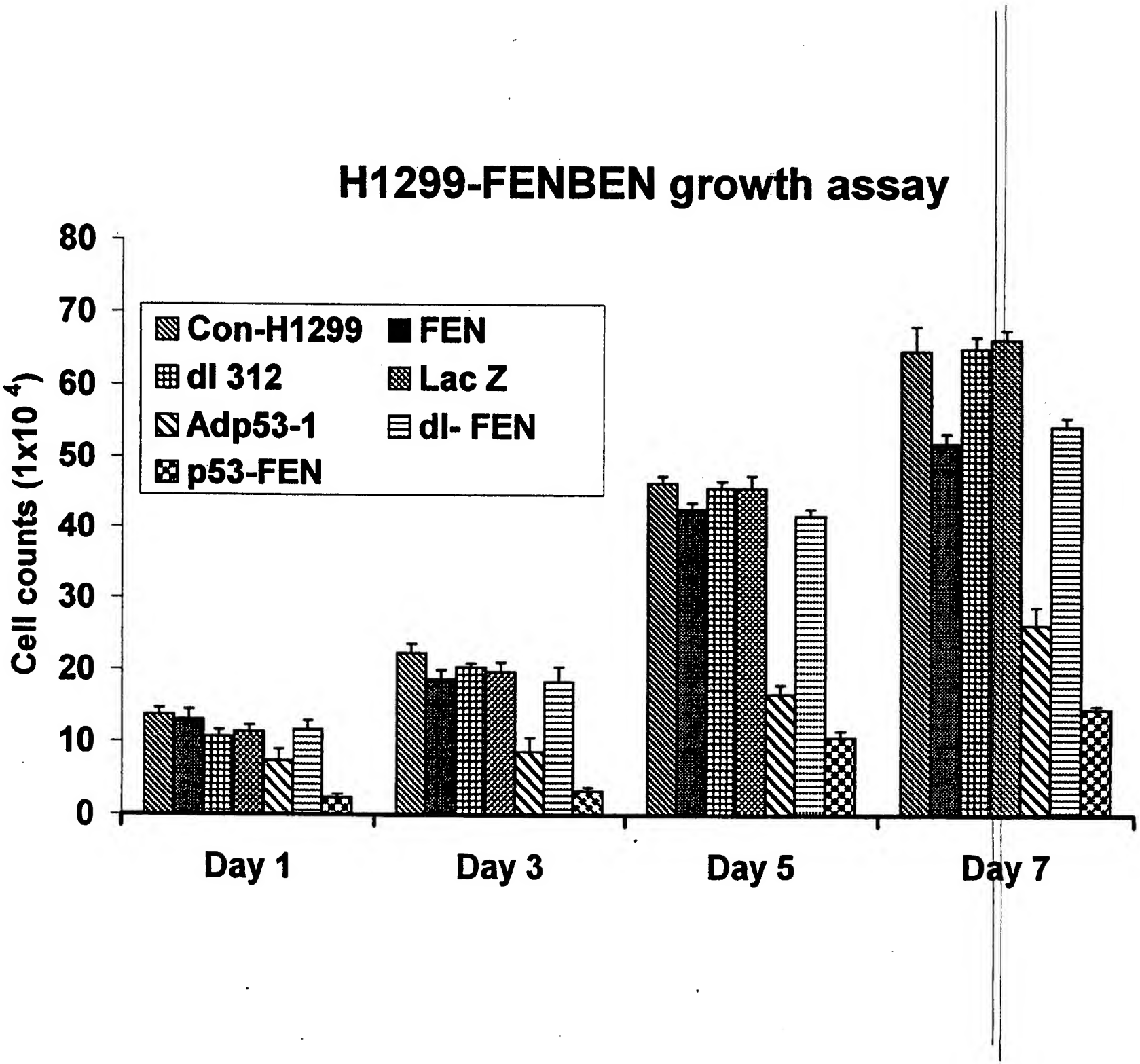

H1299-FENBEN growth assay
Con-H1299
FEN
dl 312
Lac Z
Adp53-1
dl- FEN
p53-FEN
Cell counts (1x10 4)
Day 1
Day 3
Day 5
Day 7

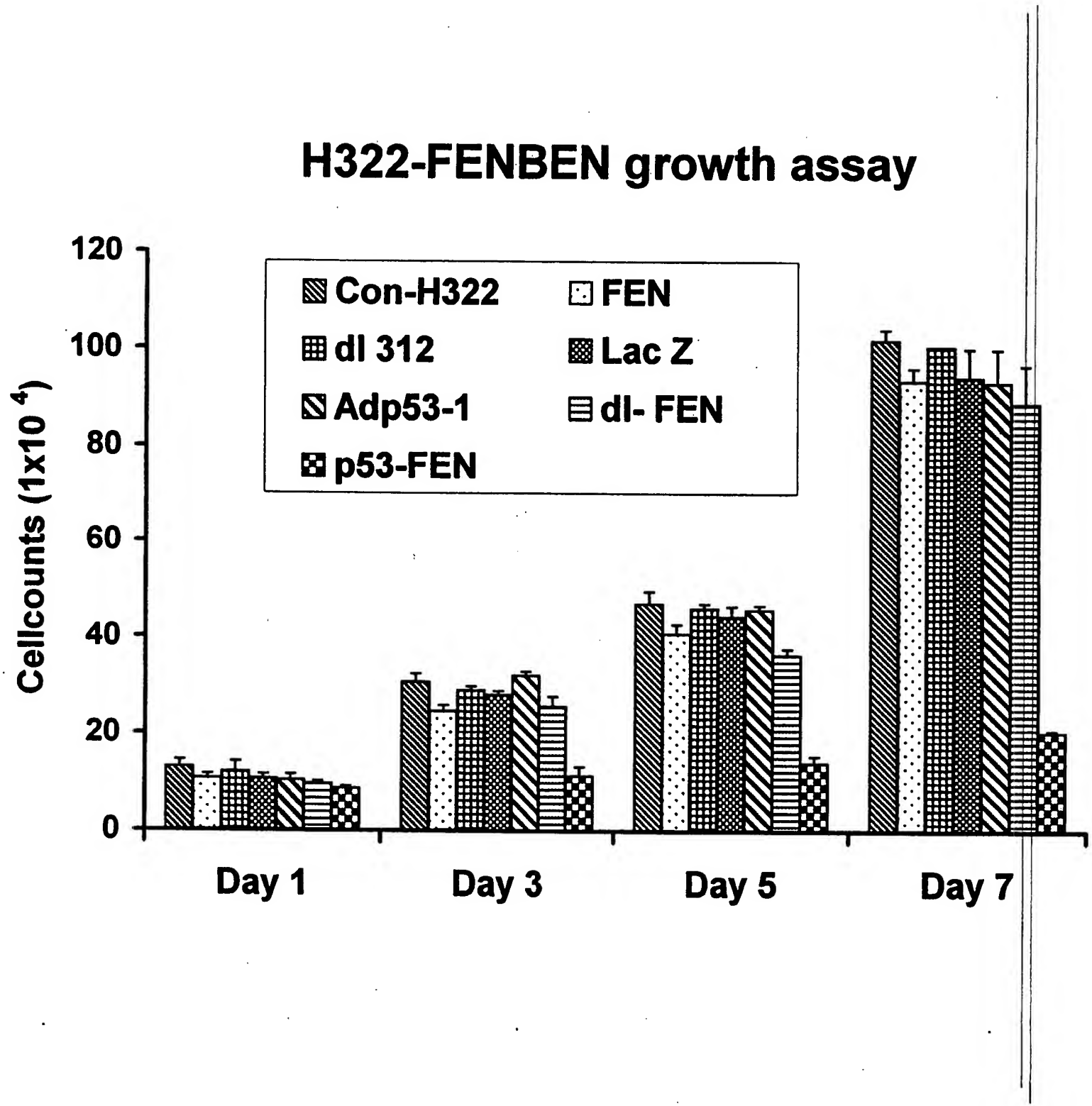

H322-FENBEN growth assay
Con-H322
FEN
dl 312
Lac Z
Adp53-1
dl- FEN
p53-FEN
Cellcounts (1x10 4)
Day 1
Day 3
Day 5
Day 7

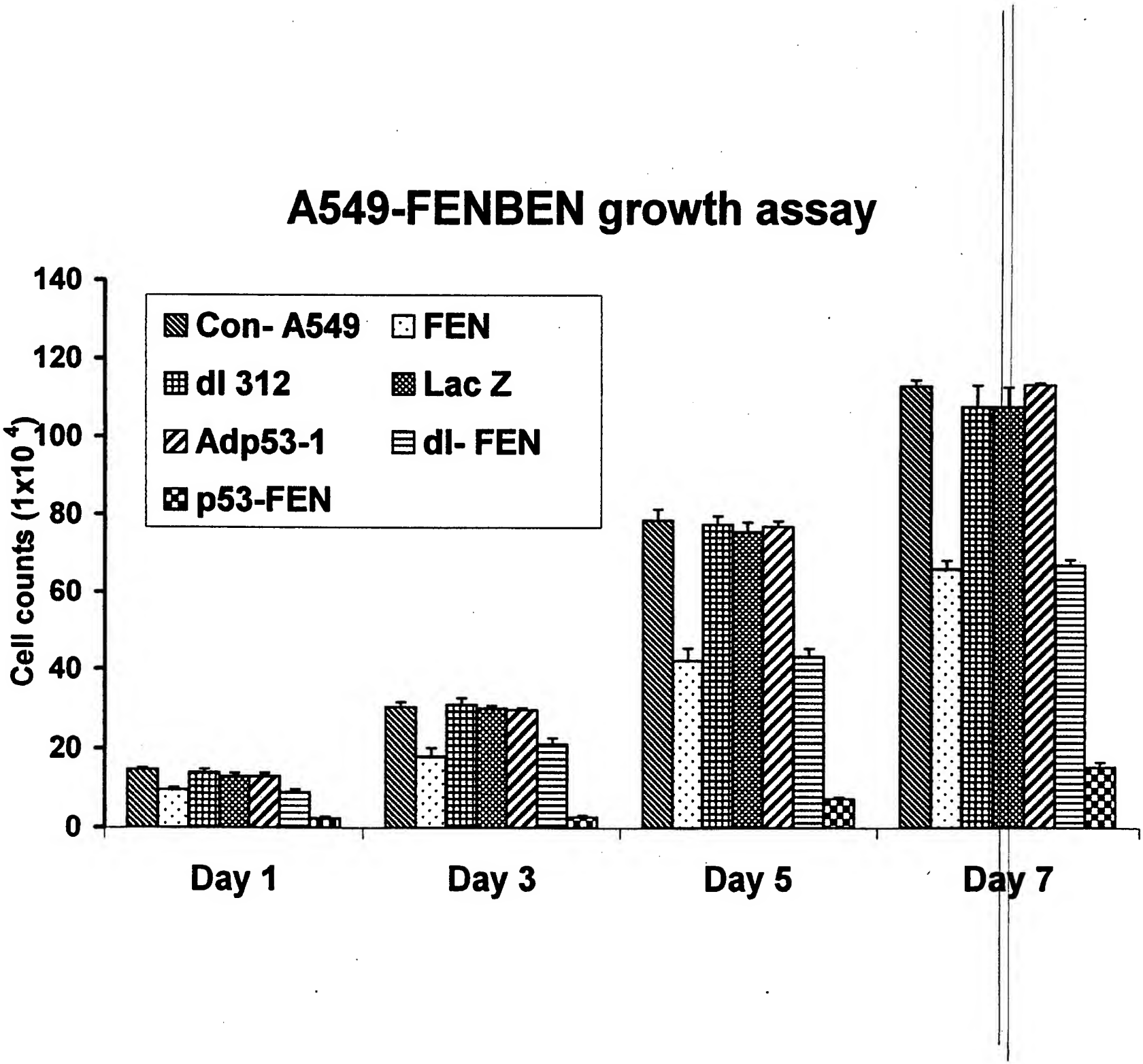

A549-FENBEN growth assay
Con- A549
FEN
dl 312
Lac Z
Adp53-1
dl- FEN
p53-FEN
Day 1
Day 3
Day 5
Day 7


H460 -FENBEN growth assay
Con-H460
FEN
dl 312
Lac Z
Adp53-1
dl- FEN
p53-FEN
Cell counts (1x10 4)
Day 1
Day 3
Day 5
Day 7

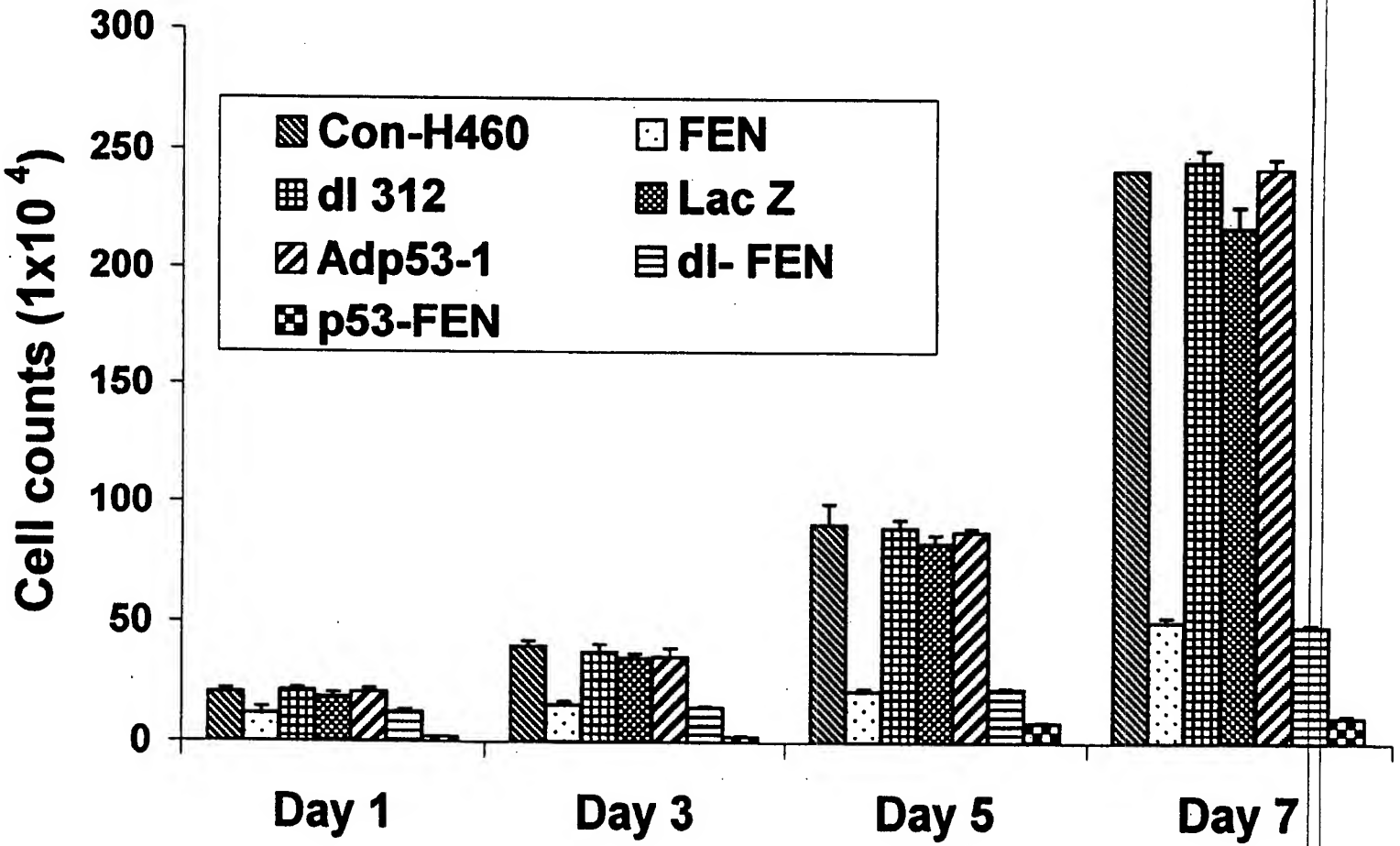

NHBEC -FENBEN growth assay
Con-NHBEC
FEN
dl 312
Lac Z
Adp53-1
dl- FEN
p53-FEN
Cell counts (1x10 4)
Day 1
Day 3
Day 5
Day 7

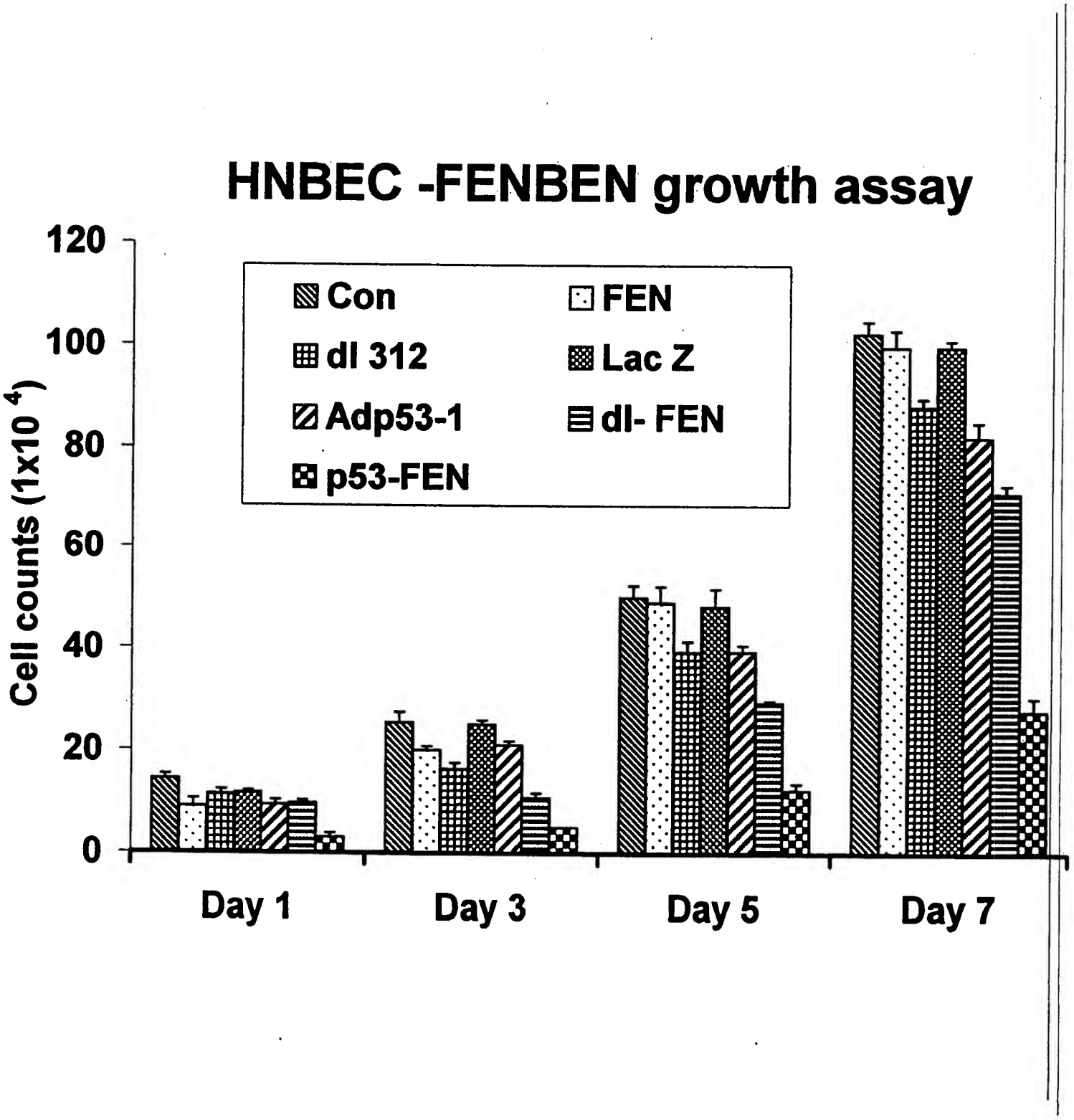

H1299-FENBEN growth assay
Cell counts (1x10 4)
80
70
60
50
40
30
20
10
0
Con-H1299
FEN
dl 312
Lac Z
Adp53-1
dl- FEN
p53-FEN
Day 1
Day 3
Day 5
Day 7


H322-FENBEN growth assay
Cellcounts (1x10 4)
120
100
80
60
40
20
0
Con-H322
FEN
dl 312
Lac Z
Adp53-1
dl- FEN
p53-FEN
Day 1
Day 3
Day 5
Day 7


A549-FENBEN growth assay
Cell counts (1x10^4)
140
120
100
80
60
40
20
0
Con- A549
FEN
dl 312
Lac Z
Adp53-1
dl- FEN
p53-FEN
Day 1
Day 3
Day 5
Day 7

H460 -FENBEN growth assay




HNBEC -FENBEN growth assay
Cell counts (1x10 4)
120
100
80
60
40
20
0
Con
FEN
dl 312
Lac Z
Adp53-1
dl- FEN
p53-FEN
Day 1
Day 3
Day 5
Day 7

NHBE

7-en 105 mg
cdw dl3 12 mol = 1
V3 mol = 1

	Day 1		Day 3		Day 5		Day 7		9	
~~control~~	x2 30	15	x2 50	25	x3 70	53	x5 79	99		
2	26	13	46	23	67	50	82	103		
3	30	15	56	28	62	44	84	105		
4	x2		x2		x3		x5			
Yeast 5	20	10	42	21	70	53	81	101		
6	14	7	40	20	65	49	76	95		
7	19	10	38	19	60	45	82	103		
8										
9	x2		x2		x3		x5			
dCSF2 10	19	10	32	16	56	42	72	90		
11	24	12	30	15	52	39	69	86		
12	23	12	35	18	50	37	70	88		
13										
14	x2		x2		x3		x5			
bSol 15	22	11	48	24	71	53	80	100		
16	24	12	52	26	62	47	81	101		
17	24	12	50	25	60	45	78	98		
18										
19	x2		x2		x3		x5			
V3 20	20	10	40	20	51	38	66	83		
21	15	8	42	21	55	41	62	78		
22	19	10	44	22	52	39	68	85		
23	x2		x2		x3		x5			
d-F 24	19	10	20	10	38	29	57	71		
25	17	9	20	10	48	30	55	69		
26	20	10	24	12	39	29	58	73		
27										
28	x1		x1		x2		x3			
29	17	4	18	5	22	11	32	24		
K-F 30	10	3	19	5	27	14	39	25		
31	9	2	19	5	24	12	40	30		

H460

		Day 1		Day 3		Day 5		Day 7	
Control	1	25	19	x3		x5		x10	
x3	2	29	22	52	39	90	88	98	245
	3	26	20	56	42	95	94	100	250
	4			50	38	92	90	92	230
Fan	5	x3		x3		x3		x5	
	6	18	14	21	16	28	21	40	50
	7	17	13	19	14	30	23	38	48
	8	10	8	22	17	27	20	42	53
	9								
dl3:2	10	x3		x3		x5		x10	
	11	30	23	54	41	72	90	96	240
	12	27	20	44	33	68	85	98	245
	13	28	21	50	38	74	93	101	252
	14								
B Rad	15	x3		x3		x5		x10	
	16	21	16	49	37	62	78	82	205
	17	27	20	47	35	68	85	90	225
	18	26	20	44	33	68	85	89	223
	19								
x3	20	x3		x3		x5		x10	
	21	30	23	52	39	71	89	95	238
	22	27	20	41	31	70	88	97	242
	23	25	19	49	37	68	85	99	248
	24								
1-F	25	x3		x3		x3		x5	
	26	15	11	20	15	29	22	38	48
	27	18	14	20	15	29	22	40	50
	28	16	12	18	14	30	23	38	48
	29	x1		x1		x2		x2	
V-F	30	7	2	7	2	15	8	20	10
	31	8	2	10	3	17	9	20	10
		6	2	9	2	15	8	24	12

H1299

	Day 1		Day 3		Day 5		Day 7	
	x2		x2		x3		x3	
1	25	13	41	24	60	45	80	60
2	30	15	42	21	62	47	88	66
3	26	13	44	22	63	47	91	68
4	x2		x2		x3		x3	
5	30	15	34	17	58	44	67	50
6	23	12	38	19	56	42	70	53
7	24	12	40	20	56	42	70	53
8								
9	x2		x2		x3		x3	
10	20	10	40	20	62	47	89	67
11	24	12	41	21	60	45	84	63
12	20	10	40	20	60	45	86	65
13								
14	x2		x2		x3		x3	
15	23	12	41	21	59	44	86	65
16	20	10	39	20	60	45	90	68
17	24	12	36	18	64	48	88	66
18								
19	x2		x2		x3		x3	
20	10	5	11	6	20	15	30	23
21	15	8	19	10	22	17	36	27
22	17	9	19	10	24	18	39	29
23								
24	x2		x2		x3		x3	
25	20	10	32	16	55	41	72	54
26	23	12	42	21	57	43	74	56
27	25	13	36	18	55	41	70	53
28	x1		x1		x2		x2	
29	9	2	10	3	20	10	30	15
30	8	2	12	3	20	10	27	14
31	13	3	16	4	24	12	29	15

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A549

	Day 1		Day 3		Day 5		Day 7		9
Control 1	x2		x3		x5		x5		
2	27	14	38	29	66	82	89	111	
3	30	15	42	32	60	75	90	113	
4	30	15	40	30	63	79	92	115	
5	x2		x3		x5		x5		
Jeh 6	17	9	25	19	35	44	51	64	
7	19	10	27	20	36	45	52	65	
8	19	10	20	15	30	38	55	69	
9									
dl3l2 10	x2		x3		x5		x5		
11	27	14	38	29	60	75	80	100	
12	26	13	44	33	62	78	88	110	
13	30	15	41	31	64	80	90	113	
14									
b-gal 15	x2		x3		x5		x5		
16	24	12	41	31	58	73	90	113	
17	28	14	38	29	63	79	87	109	
18	25	13	40	30	60	75	81	101	
19									
V3 20	x2		x3		x5		x5		
21	28	14	40	30	62	78	90	113	
22	26	13	40	30	62	78	91	114	
23	24	12	39	29	60	75	90	113	
24									
d-F 25	x2		x3		x5		x5		
26	15	8	28	21	34	43	54	68	
27	17	9	30	23	37	46	52	65	
28	20	10	25	19	33	41	54	68	
29	x1		x1		x2		x3		
V-F 30	10	3	10	3	14	4	19	14	
31	8	2	12	3	16	8	20	15	
	8	2	9	2	13	7	22	17	

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H322J

	Day 1		Day 3		Day 5		Day 7		9	
Control	x2		x3		x3		x5			
2	29	15	38	29	66	50	80	100		
3	23	12	40	30	59	44	81	101		
4	24	12	44	33	62	47	84	105		
Zero 5	x2		x3		x3		x5			
6	24	12	33	25	50	38	77	96		
7	20	10	34	26	57	43	76	95		
8	20	10	30	23	54	41	72	90		
9										
22312 10	x2		x3		x3		x5			
11	21	11	40	30	61	46	80	100		
12	24	12	41	31	63	47	80	100		
13	26	13	35	26	60	45	82	102		
14										
B-gal 15	x2		x2		x3		x5			
16	20	10	36	24	62	47	79	99		
17	20	10	37	28	58	44	78	98		
18	24	12	39	29	56	42	69	86		
19										
x3 20	x2		x3		x3		x5			
21	20	10	41	31	62	47	82	103		
22	18	9	42	32	60	45	71	89		
23	23	12	40	30	60	45	70	88		
24										
d-7 25	x2		x3		x3		x5			
26	18	9	30	23	48	36	64	80		
27	20	10	34	26	50	38	79	99		
28	20	10	37	28	46	35	70	88		
29	x2		x2		x2		x2			
X-F 30	16	8	20	10	29	15	40	20		
31	18	9	20	10	30	15	39	20		
	18	9	27	14	24	12	41	21		

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				Day 1	sd		
Con-NHB	15	13	15	14.33333	0.942809	25	23
FEN	10	7	10	9	1.414214	21	20
dl 312	10	12	12	11.33333	0.942809	16	15
Lac Z	11	12	12	11.66667	0.471405	24	26
Adp53-1	10	8	10	9.333333	0.942809	20	21
dl- FEN	10	9	10	9.666667	0.471405	10	10
p53-FEN	4	3	2	3	0.816497	5	5

				Day 1	sd		
Con-H460	19	22	20	20.33333	1.247219	39	42
FEN	14	13	8	11.66667	2.624669	16	14
dl 312	23	20	21	21.33333	1.247219	41	33
Lac Z	16	20	20	18.66667	1.885618	37	35
Adp53-1	23	20	19	20.66667	1.699673	39	31
dl- FEN	11	14	12	12.33333	1.247219	15	15
p53-FEN	2	2	2	2	0	2	3

				Day 1	sd		
Con- A549	14	15	15	14.66667	0.471405	29	32
FEN	9	10	10	9.666667	0.471405	19	20
dl 312	14	13	15	14	0.816497	29	33
Lac Z	12	14	13	13	0.816497	31	29
Adp53-1	14	13	12	13	0.816497	30	30
dl- FEN	8	9	10	9	0.816497	21	23
p53-FEN	3	2	2	2.333333	0.471405	3	3

				Day 1	sd		
Con-H322	15	12	12	13	1.414214	29	30
FEN	12	10	10	10.66667	0.942809	25	26
dl 312	11	12	13	12	0.816497	30	31
Lac Z	10	10	12	10.66667	0.942809	27	28
Adp53-1	10	9	12	10.33333	1.247219	31	32
dl- FEN	9	10	10	9.666667	0.471405	23	26
p53-FEN	8	9	9	8.666667	0.471405	10	10

				Day 1	sd		
Con-H129	13	15	13	13.66667	0.942809	24	21
FEN	15	12	12	13	1.414214	17	19
dl 312	10	12	10	10.66667	0.942809	20	21
Lac Z	12	10	12	11.33333	0.942809	21	20
Adp53-1	5	8	9	7.333333	1.699673	6	10
dl- FEN	10	12	13	11.66667	1.247219	16	21
p53-FEN	2	2	3	2.333333	0.471405	3	3

	Day 3	sd				Day 5	sd
28	25.33333	2.054805	53	50	47	50	2.44949
19	20	0.816497	53	49	45	49	3.265986
18	16.33333	1.247219	42	39	37	39.33333	2.054805
25	25	0.816497	53	47	45	48.33333	3.399346
22	21	0.816497	38	41	39	39.33333	1.247219
12	10.66667	0.942809	29	30	29	29.33333	0.471405
5	5	0	11	14	12	12.33333	1.247219

	Day 3	sd				Day 5	sd
38	39.66667	1.699673	88	94	90	90.66667	2.494438
17	15.66667	1.247219	21	23	20	21.33333	1.247219
38	37.33333	3.299832	90	85	93	89.33333	3.299832
33	35	1.632993	78	85	85	82.66667	3.299832
37	35.66667	3.399346	89	88	85	87.33333	1.699673
14	14.66667	0.471405	22	22	23	22.33333	0.471405
2	2.333333	0.471405	8	9	8	8.333333	0.471405

	Day 3	sd				Day 5	sd
30	30.33333	1.247219	82	75	79	78.66667	2.867442
15	18	2.160247	44	45	38	42.33333	3.091206
31	31	1.632993	75	78	80	77.66667	2.054805
30	30	0.816497	73	79	75	75.66667	2.494438
29	29.66667	0.471405	78	78	75	77	1.414214
19	21	1.632993	43	46	41	43.33333	2.054805
2	2.666667	0.471405	7	8	7	7.333333	0.471405

	Day 3	sd				Day 5	sd
33	30.66667	1.699673	50	44	47	47	2.44949
23	24.66667	1.247219	38	43	41	40.66667	2.054805
26	29	2.160247	46	47	45	46	0.816497
29	28	0.816497	47	44	42	44.33333	2.054805
33	32	0.816497	47	45	45	45.66667	0.942809
28	25.66667	2.054805	36	38	35	36.33333	1.247219
14	11.33333	1.885618	15	15	12	14	1.414214

	Day 3	sd				Day 5	sd
22	22.33333	1.247219	45	47	47	46.33333	0.942809
20	18.66667	1.247219	44	42	42	42.66667	0.942809
20	20.33333	0.471405	47	45	45	45.66667	0.942809
18	19.66667	1.247219	44	45	48	45.66667	1.699673
10	8.666667	1.885618	15	17	18	16.66667	1.247219
18	18.33333	2.054805	41	43	41	41.66667	0.942809
4	3.333333	0.471405	10	10	12	10.66667	0.942809

			Day 7	sd
99	103	105	102.3333	2.494438
101	95	103	99.66667	3.399346
90	86	88	88	1.632993
100	101	98	99.66667	1.247219
83	78	85	82	2.94392
71	69	73	71	1.632993
24	29	30	27.66667	2.624669

			Day 7	sd
245	250	230	241.6667	8.498366
50	48	53	50.33333	2.054805
240	245	252	245.6667	4.921608
205	225	223	217.6667	8.993825
238	242	248	242.6667	4.109609
48	50	48	48.66667	0.942809
10	10	12	10.66667	0.942809

			Day 7	sd
111	113	115	113	1.632993
64	65	69	66	2.160247
100	110	113	107.6667	5.557777
113	109	101	107.6667	4.988877
113	114	113	113.3333	0.471405
68	65	68	67	1.414214
14	15	17	15.33333	1.247219

			Day 7	sd
100	101	105	102	2.160247
96	95	90	93.66667	2.624669
100	100	102	100.6667	0.942809
99	98	86	94.33333	5.906682
103	89	88	93.33333	6.847546
80	99	88	89	7.788881
20	20	21	20.33333	0.471405

			Day 7	sd
60	66	68	64.66667	3.399346
50	53	53	52	1.414214
67	63	65	65	1.632993
65	68	66	66.33333	1.247219
23	27	29	26.33333	2.494438
54	56	53	54.33333	1.247219
15	14	15	14.66667	0.471405

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